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The Janus effect: two faces of aldosterone

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Inhibition of the nitric oxide pathway by N^{ω}-nitro-L-arginine methyl ester (L-NAME) is well known to produce hypertension and proteinuria, but the mechanisms are less straightforward. Prolonged administration of mineralocorticoids mimics the pathological findings produced by L-NAME. Ikeda and colleagues provide a clue to the mechanism by showing that exposure to L-NAME increases plasma aldosterone 50-fold, and that spironolactone markedly attenuates the renal changes. Thus, chronic L-NAME exposure may turn out to be a model of ineralocorticoid excess.

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In this issue of *Kidney International*, Ikeda and colleagues[1] present a series of experiments conducted in a previously well-described rat model of renal injury. With the use of an inhibitor of nitric oxide production, N^{ω}-nitro-L-arginine methyl ester (L-NAME), rats develop hypertension with proteinuria and evidence of renal fibrosis over a period of 12 weeks. The authors present evidence that aldosterone mediates some of the renal injury in this model in addition to angiotensin II, a finding consistent with the literature.[2–4] Treatment with L-NAME results not only in the development of hypertension and signs of renal fibrosis but in a more than 50-fold increase in plasma aldosterone. Blocking aldosterone action with spironolactone attenuates the pathological findings, largely independently of any effect on blood pressure or dietary salt intake. The contribution of Ikeda *et al.*[1] lies not in producing another hypertension–renal injury model but in providing new clues to the hormones and pathways governing renal injury and fibrosis.

Ikeda and colleagues[1] show more than that aldosterone antagonists attenuate renal damage brought on by L-NAME; their findings support a physiological and probably important connection between nitric oxide metabolism and the generation of aldosterone. Others have previously suggested that nitric oxide acts to suppress aldosterone secretion directly by reducing steroidogenesis in zona glomerulosa cells and by downregulating angiotensin I receptors, both effects independent of guanylyl cyclase.[5–8] Using L-NAME to block nitric oxide production leads to a profound increase in plasma aldosterone levels in the model of Ikeda *et al.*[1] and makes one wonder whether the effects observed are as much the result of prolonged mineralocorticoid excess as the result of nitric oxide inhibition.

Aldosterone has always fascinated students of renal physiology, and the hormone has an established role as one of the guardians of the composition of the body fluids, or Claude Bernard's *milieu intérieur*. Early experiments using the toad urinary bladder and, later, mammalian renal collecting duct cells clearly showed that aldosterone induced transepithelial sodium reabsorption.[9] The sodium transport required a specific receptor, which, when bound to the hormone, translocated to the cell nucleus and eventually led to the generation of new proteins. Seminal studies later followed that described aldosterone's role in renal potassium and hydrogen ion secretion. Still later, syndromes of mineralocorticoid excess appeared in the clinical literature and proved the point that too much of a good thing could produce electrolyte disturbances and hypertension.

Early on, investigators described a curious paradox regarding aldosterone and its actions on the kidney. The effect of aldosterone on sodium transmembrane transport was easy to demonstrate in cell membrane preparations such as the toad bladder and isolated perfused renal collecting ducts, yet aldosterone's influence on renal sodium reabsorption in adrenally intact animals could not be reliably reproduced.[10] However, animals adrenalectomized before treatment with physiological doses of aldosterone clearly showed the expected sodium reabsorptive response to the hormone. This was the first evidence that aldosterone itself was physiologically tightly regulated by naturally occurring endogenously generated factors likely emanating from the adrenal gland.

Uete and Venning first described candidate adrenal steroids that might interfere with the renal antinatriuretic actions of mineralocorticoids.[11] Administration of cortisol or its 11-dehydro meta-bolite cortisone attenuated the antinatriuretic response to both deoxycorticosterone acetate and aldosterone in the adrenalectomized rat model. This blunting effect of cortisol and cortisone was dose dependent. Later, Alberti and Sharp expanded prior observations and provided

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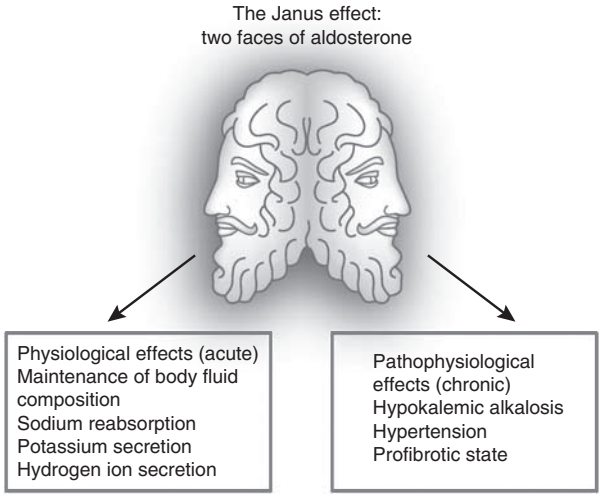

Figure 1 | Two faces of aldosterone. In Roman mythology, Janus was the god of the gates or doorways to the home. His simultaneous gaze in two directions signified a look toward the beginning and end or a view at opposites. Aldosterone has an acute or semi-acute (minutes to hours) physiological function to regulate the composition of body fluids. Prolonged or excess exposure to the mineralocorticoid (days, weeks, months) produces a pathological state leading to organ fibrosis and hypertension.

a mechanism by demonstrating that cortisone functioned as an aldosterone antagonist.[12] Cortisone had no direct effect on sodium transport by itself, but it was able to displace aldosterone from its receptor.

Our laboratories reexamined the Alberti and Sharp experiment, first using the toad urinary bladder as a model of the mammalian collecting duct.[13] The toad bladder contains mineralocorticoid receptors and an NAD-dependent unidirectional form of 11β-hydroxysteroid dehydrogenase (11β-HSD). Toad bladders exposed to 11-dehydrocorticosterone and then stimulated with aldosterone exhibited a markedly suppressed response in transepithelial sodium transport compared with controls stimulated with aldosterone alone. A similar pattern was seen when bladders were exposed to corticosterone and then stimulated with aldosterone. Both corticosterone and 11-dehydrocorticosterone also suppressed aldosterone-induced renal sodium retention in the adrenalectomized rat model.[14] Lastly, in studies performed with ME Oblin, we have shown that 11-dehydrocorticosterone alone does not directly activate mineralocorticoid receptors, but it is able to blunt aldosterone activation of these same receptors.[15] As an aside, 11-dehydrocorticosterone has no effect on dexamethasone binding to glucocorticoid receptors. Odermatt and colleagues extended these findings using transfected HEK-293 cells by demonstrating that both 11-dehydrocorticosterone and cortisone in concentrations as low as 50 nM block aldosterone (10 nM) induced mineralocorticoid receptor activation.[16]

Aldosterone's second face, as a profibrotic factor, is a more recent concept (Figure 1). First reports focused on aldosterone promoting fibrotic changes in the heart and contributing to heart failure.[17] Later, aldosterone as a fibrotic factor was implicated in the response to injury in various models of kidney injury, in addition to elements of the renin–angiotensin system. Investigators are now beginning to explore all the various pathways, where mineralocorticoids participate in the injury cascade. That aldosterone may be part of the fibrotic response to injury is interesting enough but begs the question: could excess aldosterone exposure itself cause injury?

Aldosterone functions as a profibrotic agent through a multitude of pathways well summarized elsewhere. Major organ targets for aldosterone-induced fibrosis, including the heart, vascular tissue, and the kidney, express mineralocorticoid receptors; cells and tissues devoid of mineralocorticoid receptors are likely immune to the fibrotic effects of the hormone. As with electrolyte transport, aldosterone activation of serum glucocorticoid kinase (SGK) appears to be part of the initial phase of the profibrotic process. Fibrosis involves a response to inflammation followed by activation of certain growth factors leading to fibroblast growth and net matrix production. Aldosterone seems to be active in all phases of this cascade;[18] the hormone can stimulate reactive oxygen species, induce transforming growth factor-β and connective tissue growth factor, and both increase the synthesis of extracellular matrix and impair matrix breakdown.

The previously mentioned tight regulation of aldosterone-stimulated electrolyte transport may also limit the pathophysiological profibrotic effects of the mineralocorticoid. In preliminary studies, we have observed that cultured mouse renal inner medullary collecting duct cells, which contain mineralocorticoid receptors, increase collagen production after being exposed to 10 nM aldosterone for a minimum of 48 h. Immortalized rat renal proximal tubular cells (IRPTCs) do not produce more collagen following prolonged aldosterone exposure, and IRPTCs do not contain mineralocorticoid receptors. Both 1 μM spironolactone and 1 μM 11-dehydrocorticosterone effectively prevent this increase in collagen generation. Given their suppressive effects on aldosterone-induced electrolyte transport and now on collagen production, the 11-keto glucocorticoid metabolites generated by renal 11β-hydroxysteroid dehydrogenase may function as naturally occurring endogenously produced spironolactones.

From our own experiments and now from those of Ikeda and colleagues,[1] it is clear that aldosterone-induced fibrotic changes develop over time. Acute mineralocorticoid secretion and action satisfy the needs for sodium conservation and potassium secretion; only prolonged and/or unregulated mineralocorticoid exposure produces fibrotic changes in responsive tissues. One could conclude from Ikeda *et al.*[1] that the nitric oxide system also plays a vital role regulating the many enigmatic aldosterone-activated pathways.

DISCLOSURE

The author declared no competing interests.

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Improving clinical trial design for inquiries into the mechanisms of cyst growth in ADPKD

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Accurate and reliable estimates of kidney and cyst volume, the hallmark of disease progression in ADPKD are now available. These powerful and exciting tools make it possible to consider both short and long term randomized clinical trials in ADPKD at various stages of disease. Highlights of the work by Kistler and colleagues are now provided.

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Accuracy, precision, and reliability of renal and cyst volume measures in human ADPKD

Estimates of total renal and cyst volume based on magnetic resonance (MR) without gadolinium demonstrate high accuracy, precision, and reliability throughout a broad range of renal volumes in autosomal dominant polycystic kidney disease (ADPKD). Kistler and colleagues[1] (this issue) present a meticulous and elegantly executed study of MR-based renal and cyst volume estimates over time in human ADPKD. Manual measurements with trained readers demonstrated >0.99 concordance coefficients for inter- and intrareader variability for total renal volume and slightly less (0.944) for total cyst volume. These findings are similar to those of the Consortium for Radiologic Imaging Studies in Polycystic Kidney Disease (CRISP) study[2] (inter-reader variability 2.1% and intrareader variability 2.4% for renal volume), in which gadolinium was used. High accuracy, precision, and reliability without gadolinium enhancement allow for the benefit of reduced cost of imaging and remove the potential risk of developing nephrogenic systemic fibrosis.

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Renal volume measurements in the study by Kistler *et al.*[1] were completed with the use of manual segmentation by trained personnel supported with software. The investigators acknowledge that manual segmentation requires significant effort and is therefore restricted from widespread use. Manual segmentation precludes clinical implementation for patients with ADPKD, and a high priority to develop sophisticated image analysis software to support more automated, accurate and reliable renal and cyst volume measurement is needed in ADPKD. Development of such technology would allow clinicians to focus on patients at high risk for progression to renal failure and provide valuable predictive information to their patients.

Cyst volume measurements in this study were performed using stereological methods in T2-weighted images, similar to the methods used for renal volume estimates in the CRISP study.[2] Intra- and interobserver variability was similar to that found in CRISP, without the use of gadolinium. However, estimations of noncystic parenchyma without gadolinium are not feasible, and this may be an important consideration in the evaluation of disease progression in ADPKD.[3] In addition, estimates of renal function, using contrast-enhanced MR imaging, a novel functional application of renal MR imaging,[4] are also not possible in this setting.